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(54) Bone regeneration material

(57) Provided is a bone regeneration material for expediting formation of a new bone tissue, wherein a polyphosphoric acid and/or a polyphosphate is contained in a material having a biocompatibility, a filler for cosmetic surgery or a bone morphogenetic protein.

This bone regeneration material can expedite new bone formation and shorten a time that lapses until the healing or the restoration in the treatment of bone fracture owing to a physical shock or of damage of a bone accompanied by surgical operation.

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Description

Field of the Invention

[0001] The present invention relates to a bone regeneration material for expediting formation of a new bone tissue. More specifically, the invention relates to a bone regeneration material comprising inorganic polyphosphate.

Description of the Related Art

[0002] A bone is a specialized and hardened connective tissue that is composed of cells and an extracellular matrix, and it is differents from other connective tissue in that matrix of the bone is mineralized. The mineral is calcium phosphate which is a hydroxyapatite crystal ($Ca_{10}(PO_4)_6(OH)_2$). The bone is an extremly hard tissue capable of providing support and protection from physical stress. Accordingly, the reduction or the damage of the bone owing to the fracture or the pathological change leads to the disability, the waste of time and money. When the bone is removed by any reasons, the defected bone need to be generated as soon as possible.

[0003] But if regeneration of the defect cannot occur, replacement of the defect by an artificial bone or by bones from other parts of the body must be operated.

[0004] Further, in the treatment of the damage (fracture) of the bone by the physical shock or the damage of the bone accompanied by the surgical operation, the artificial middle setting of various auxiliary bones including artificial bones and immobilization or fixation of fractured portions of bones have been conducted.

[0005] It takes much time until the bone restores the original shape and function, and the physical and mental stresses of patients are considerably great. Further, the longer the process to healing, the greater the opportunity in which patients might be exposed to bacterial infection. There is a fear that the damaged portions might not be healed completely. Regarding the existing selective materials for increase and regeneration of the bone, various materials which can function as artificial fillers for bone restoration, such as bioceramics, composite materials, bone morphogenetic materials, and natural and synthetic polymers have been studied.

[0006] In case of teeth, replacement and reconstruction of fractured, lost, and any pathologically or physiologically wounded bony parts in the oro-maxillofacial region are also important in many aspect. In particular, an alveolar bone supporting the teeth is vulnerable to bacterial infection. Once the alveolar bone is infected or destroyed, it can hardly be restored to the original level by itself. Generally, implantation for constructing an artificial tooth by inserting a metallic implant member in which titanium is used as a base material into a jaw bone is useful with respect to the loss of an inherent tooth. However, this implantation technique is unsatisfactory for supporting the portion of the body around the implanted part, and causes an excess occlusal force to the adjacent bony structure. Thus, this has not always been conducted successfully.

[0007] As a curing method to solve these problems, there is a method for accelerating regeneration of a tissue and a bone. In order to accelerate regeneration of a bone in a defected bony area, a demineralized bone, hydroxyapatite or the other implant substitute has been used. However, no satisfactory effect has been provided.

[0008] For an ideal filler or implant material, properties such as a biocompatibility, a bactericidal activity, a bone morphogenetic activity (stimulus for phenotypically converting mesenchymal cells into osteoblasts in bone formation) and a bone conductive activity (which acts as a lattice for new bone formation) are required.

[0009] Further, it is advisable that a filler is biodegradable, free from immunogenicity and non-toxic in view of the somatic tissue. However, there is actually no material that meets all of these requirements. Only a material that meets some of these requirements exists.

Summary of the Invention

[0010] It is an object of the invention to provide, for overcoming the defects associated with the conventional bone curing means or substitute bone materials, a bone regeneration material which can expedite new bone formation and shorten a time that lapses until the healing or the restoration.

[0011] That is, the invention relates to a bone regeneration material for expediting formation of a new bone tissue, the bone regeneration material containing a linear condensed polyphosphoric acid or/and a polyphosphate. Further, the invention relates to a bone regeneration material for expediting regeneration of a fractured bone wherein a polyphosphate is contained in a substrate composed of a material having a biocompatibility.

[0012] Still further, the invention relates to a bone regeneration material for expediting formation of a new bone tissue wherein a polyphosphoric acid is contained in a filler for cosmetic surgery.

[0013] Furthermore, the invention relates to a bone regeneration material wherein a polyphosphoric acid is mixed with a bone morphogenetic protein or/and a natural substance containing a bone morphogenetic factor.

Brief Description of the Drawings

[0014]

Fig. 1 is a stereoscopic microphotograph of a hole of a thighbone after one week of the treatment with a collagen tape immersed in sterile water.
Fig. 2 is a stereoscopic microphotograph of a hole of a thighbone after one week of the treatment with a collagen tape immersed in a polyphosphoric acid aqueous solution.
Fig. 3 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after one week of the treatment with a collagen tape immersed in sterile water.
Fig. 4 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after one week of the treatment with a collagen tape immersed in a polyphosphoric acid aqueous solution.
Fig. 5 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after two weeks of the treatment with a collagen tape immersed in sterile water.
Fig. 6 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after two weeks of the treatment with a collagen tape immersed in a polyphosphoric acid aqueous solution.
Fig. 7 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after two weeks of the treatment with a dust bone immersed in sterile water.
Fig. 8 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after two weeks of the treatment with a dust bone immersed in a polyphosphoric acid aqueous solution. Detailed Description of the Invention.

[0015] The polyphosphoric acid includes a linear condensed polyphosphoric acid obtained through dehydrocondensation of an orthophosphoric acid, a side chain polyphosphoric acid in which an organic group is introduced into a side chain, and a cyclic polyphosphoric acid. Especially preferable is a linear condensed polyphosphoric acid represented by formula $(P_nO_{3n+1})_{(n+2)}H$ and having a structure that two or more PO_4 tetrahedrons are linearly bound with a top oxygen atom held in common. A polyphosphate is a compound having a molecular structure that hydrogen of a hydroxyl group of a polyphosphoric acid is replaced with a metal.

[0016] Examples of the metal include sodium and potassium. n is an integer of at least 2, and it is preferably between 5 and 5,000, more preferably between 15 and 2,000. Examples of the substrate composed of the material having the biocompatibility include a synthetic polymer having a biocompatibility, and a sheet, a film, a fiber and a porous material made of a natural material. The polyphosphoric acid is used by being mixed with these materials, by being coated on the surface of the substrate or by being dipped in fibers or a porous material. Examples of the synthetic polymer include non-bioabsorbable polymers such as polypropylene, polyethylene, polyvinyl chloride, polyester, polycarbonate, cellulose, polyamide, polyfluoroethylene and polyurethane; and bioabsorbable polymers such as polyglycolic acid, polylactide, collagen, polyvinyl alcohol, polyvinyl pyrrolidone, polyamino acid, polycaprolactone, polydioxane and a copolymer of vinyl acetate and an unsaturated carboxylic acid. In the bone regeneration material, the polyphosphoric acid is contained in the material having the biocompatibility. The amount of the polyphosphoric acid contained in the material having the biocompatibility is between 0.001 and 20% by weight, preferably between 0.005 and 10% by weight, more preferably between 1 and 5% by weight. When the amount of the polyphosphoric acid exceeds 20% by weight, cells tend to undergo necrosis. When it is less than 0.001% by weight, the effect of the bone regeneration is decreased.

[0017] Further, the filler for cosmetic surgery is an artificial bone component used as a bone filler in the cosmetic surgery region. Examples of the filler include hydroxyapatite, calcium secondary phosphate, calcium tertiary phosphate and calcium quaternary phosphate. Examples of the bone morphogenetic protein include bone morphogenetic proteins such as BMP-1, BMP-2 and BMP-3, transforming growth factors such as TGF-b, osteopontin and osteocalcin. Further, examples of the natural substance containing a bone morphogenetic factor include crushed animal bone, mineral-defective bone substrate and the like. The natural substance containing a bone morphogenetic factor is mixed with a polyphosphoric acid in combination with the bone morphogenetic protein, and the mixture can locally be administered as an implant or a device. In this case, the product to be administered is occluded or injected in a physiologically acceptable viscous form free from a pyrogenic substance and suitable for feeding into a fractured bone site. Consequently, a hard or soft bone structure is formed in the fractured bone site, providing a matrix which can be re-absorbed into the body in an optimum state.

[0018] The bone regeneration material of the invention is used, as an osterogenic preparation containing the polyphosphoric acid, in the preventive applications such as improvements of the reduction of the occlusive fracture or the complicated fracture and the fixation of artificial joints. Further, the osterogenic preparation newly induces the bone formation, and it is used in the restoration of the innate or traumatic defective portion or the defective portion caused by tumor incision, and in the treatment of the cosmetic surgery, the treatment of the periodontal disease or the other dental restoration process. Moreover, with respect to the bone regeneration material of the invention, the material containing

the polyphosphoric acid is used by being coated on the surface of the substrate composed of the material having the biocompatibility, such as a sheet, a film, fibers or a porous material.

[0019] The bone regeneration material of the invention can expedite new bone formation and shorten a time that lapses until the healing or the restoration in the treatment of bone fracture owing to a physical shock or of damage of a bone accompanied by surgical operation.

Examples

[0020] The present invention is illustrated specifically by referring to the following Examples.

Example 1

[0021] A white rabbit (from New Zealand, body weight 3 kg) was subjected to anesthetic injection, and the thighbone thereof was exposed. Two holes were formed in the neck of the thighbone and near the joint cone, an end of the thighbone using a sterilized drill (3 mm in diameter) until the tip of the drill reached the soft tissue of the thighbone. A collagen tape (CollaTape, supplied by Calcitec) having a size of 1 cm x 1 cm was immersed in 30 ml of a 2% sodium polyphosphate (average chain length 75) aqueous solution, and sterilely dried. This collagen tape was embedded in the hole formed in the thighbone on the right leg of the white rabbit.

[0022] Further, a collagen tape immersed in sterile water as a control was embedded in the hole formed in the thighbone on the left leg of the white rabbit. In this state, the incised portions were sutured, and the conditions of the new bone generation in the holes of the thighbone after 1 and 2 weeks were observed. The thighbones extracted for observation were immobilized with 10% formalin.

[0023] Figs. 1 and 2 show states of the holes, after 1 week, which were cut longitudinally, as observed with a stereoscopic microscope. Fig. 1 shows a portion treated with the collagen tape immersed in sterile water. Fig. 2 shows a portion treated with the collagen tape immersed in the polyphosphoric acid aqueous solution. With the collagen tape immersed in sterile water as shown in Fig. 1, no new bone formation was observed at all. Meanwhile, with the collagen tape immersed in the polyphosphoric acid aqueous solution as shown in Fig. 2, it was identified that the new bone in the considerable amount was formed around the bottom edge of the hole, and it was extended to the soft tissue.

[0024] A part of the tissue sample in each hole of the thighbone was taken out for histologically observing the state of the new bone formation in the hole of the thighbone after 1 and 2 weeks using the above-mentioned collagen tapes immersed in sterile water and in the polyphosphoric acid aqueous solution. This tissue sample was treated with 10% EDTA for 2 months to conduct decalcification. The sample decalcified was dehydrated with ethanol at various concentrations and finally with xylene, and wrapped in paraffin. The thus-wrapped sample was cut to a thickness of 5 mm, stained with Azan, and observed under a microscope. With respect to the tissue condition after 1 week, the sample treated with the collagen tape immersed in sterile water is shown in Fig. 3, and the sample treated with the collagen tape immersed in the polyphosphoric acid aqueous solution in Fig. 4 respectively.

[0025] In the sample treated with the collagen tape immersed in sterile water, as is clear from Fig. 3, the collagen tape (C) was covered with a fibrous tissue (F), and the trabecular bone (TB) derived from the endosteum of the compact bone (B) was approaching to the fibrous tissue. On the other hand, in the sample treated with the collagen tape immersed in the polyphosphoric acid aqueous solution, as shown in Fig. 4, most of the collagen tape was absorbed, and replaced with the fibrous tissue (F). Further, a mass (*) of an immature fibrous trabecular bone derived not from the compact bone (B) but from a new fibrous tissue in the hole could already be identified in six positions of the fibrous tissue.

[0026] Next, with respect to the state after 2 weeks, the sample treated with the collagen tape immersed in sterile water is shown in Fig. 5, and the sample treated with the collagen tape immersed in the polyphosphoric acid aqueous solution in Fig. 6 respectively. In the sample treated with the collagen tape immersed in sterile water, as shown in Fig. 5, both ends of the bone were connected by means of the trabecular bone (TB), but the collagen tape (C) was not completely absorbed, and the trabecular bone (TB) derived from the endosteum surrounded the collagen tape (C) to form a callus. Whereas, the sample treated with the collagen tape immersed in the polyphosphoric acid aqueous solution, as shown in Fig. 6, the collagen tape was absorbed almost completely, and could not be observed. Further, the trabecular bones, primary bones, which were newly formed, were bound to each other, and replaced with the collagen tape. The trabecular bones (*) derived from the new fibrous tissue and replaced with the collagen tape were connected with the trabecular bone derived from the endosteum, and the calluses were formed with the two trabecular bones of the different origins. These restored the holes in such a manner that both ends of the bone were connected therewith.

Example 2

[0027] Holes were formed in the thighbone of the white rabbit in the same manner as in Example 1. Ten milligrams of

a human dust bone (particle diameter from 250 to 300 mm) demineralized was immersed in 30 ml of a 2% sodium polyphosphate (average chain length 75) aqueous solution, and sterilely dried. This dust bone was packed in the hole formed in the thighbone on the right leg of the white rabbit. Further, a dust bone immersed in sterile water as a control was packed in the hole formed in the thighbone on the left leg of the white rabbit. In this state, the incised portions were sutured, and the state of the new bone formation in the holes of the thighbone after 2 weeks was histologically observed in the same manner as in Example 1. Fig. 7 shows the results of the electron microscope of the sample treated with the dust bone immersed in sterile water. The hole was mainly filled with the dust bone (DB) and the fibrous tissue (F), and the new trabecular bone was observed only thinly. Fig. 8 shows the results of the electron microscope of the sample treated with the dust bone immersed in the polyphosphoric acid aqueous solution. The hole was filled with the new trabecular bone (*) and the dust bone (DB).

Example 3

[0028] Holes were formed in the thighbone of the white rabbit in the same manner as in Example 1. A collagen tape was immersed in 30 ml of a 2% aqueous solution of sodium polyphosphate (Polyphosphate glass, supplied by Sigma) having various chain lengths, and sterilely dried. The collagen tape was embedded in the hole formed in the thighbone on the right leg of the white rabbit. Further, a collagen tape immersed in sterile water as a control was embedded in the hole formed in the thighbone on the left leg of the white rabbit. In this state, the incised portions were sutured, and the state of new bone formation in the holes of the thighbone after 2 weeks was histologically observed. The chain lengths of sodium polyphosphate used were, in terms of the phosphoric acid group, (1) an average chain length of 15 ($Na_{17}P_{15}O_{46}$), (2) an average chain length of 25 ($Na_{27}P_{25}O_{76}$), (3) an average chain length of 35 ($Na_{37}P_{35}O_{106}$) and (4) an average chain length of 65 ($Na_{67}P_{65}O_{196}$).

[0029] In the experiments using all of polyphosphoric acids, the new bone formation was accelerated without being influenced with the chain lengths of the polyphosphoric acids.

Example 4

[0030] To observe a direct effect of polyphosphate on an osteogenic cell which involves in bone formation, cell activity of MC3T3-E1 osteogenic cell originated from a mouse calvarium was determined by MTT(3-[4,5-dimethylthyazol-2-yl]-2,5-diphenyltetrazoium bromide) assay in the presence of polyphosphate with a chain length of 75 at various concentrations.

[0031] First, the osteogenic EI cell was grown in α-Minimal Essential Medium (α-MEM; Gibco, U.S.A.) supplemented with 10% fetal bovine serum(FBS)at 37 °C with 5% CO_2. The grown cells were distributed in each well of a 24-well plate, adjusting the cell number to 5×10^4 cells per ml and incubated in the same medium for 1 day. The cells were further incubated in the medium without FBS overnight, undergoing their Go stage. After removing the cultured medium, the cells were incubated with increasing concentrations (final concentrations of 0.001~0.01%) of polyphosphate in a total of 1 ml of α -MEM for 24 hours. Instead of polyphosphate, 10 μl of distilled water or α-MEM with 10% FBS was included in the culture as control. After, discarding the culture supernatant, 450 μl of α-MEM without FBS and 50 μl of MTT(50 mg/ml: Acros Organics, Belgium) were incubated with the cells for 4 hours at 37°C with 5 % CO_2. The supernatant was removed and then the cells treated with 500 μl of isopropanol containing 0.04N HCl. The resulting mixture was collected and measured its MTT activity colorimetrically at 570nm as compared at 630nm as reference.

[0032] The MTT activity of MC3T3-EI osteogenic cell at Go stage was increased by approximately 27% in the presence of polyphosphate at the concentrations of 0.001 ~0.0025% ; it was 30% of the increased activity by FBS which contains a variety of growth factors. The activity was gradually decreased down to the control level as concentrations of polyphosphate increased (Table 1).

Table 1

MTT activity of MC3T3-EI osteogenic cell in the presence of polyphosphate with a chain length of 75		
Activators	Absorbance(A570nm-A630nm)	Relative percent
Distilled water	0.223	100.0
Fetal bovines serum(10%)	0.427	191.5
Polyphosphate(0.001%)	0.282	126.5

Table 1 (continued)

MTT activity of MC3T3-EI osteogenic cell in the presence of polyphosphate with a chain length of 75		
Activators	Absorbance(A570nm-A630nm)	Relative percent
Polyphosphate(0. 0025%)	0.284	127.4
Polyphosphate(0.005%)	0.260	116.6
Polyphosphate (0.0075%)	0.238	106.7
Polyphosphate(0.01%)	0.222	99.6

Claims

1. A bone regeneration material for expediting formation of a new bone tissue, which material contains a polyphosphoric acid.

2. The bone regeneration material as claimed in claim 1, wherein the polyphosphoric acid is a linear condensed polyphosphoric acid.

3. The bone regeneration material as claimed in claim 1 or 2, wherein the polyphosphoric acid is a polyphosphate.

4. The bone regeneration material as claimed in any one of claims 1 to 3, wherein the polyphosphoric acid is represented by formula $(P_nO_{3n+1})_{(n+2)}$ - which n is an integer of 2 or more.

5. The bone regeneration material as claimed in any one of claims 1 to 4, wherein the polyphosphoric acid is contained in a substrate composed of a material having a biocompatibility.

6. The bone regeneration material as claimed in claim 5, wherein the polyphosphoric acid is contained in an amount of from 0.001 to 20% by weight in the material having the biocompatibility.

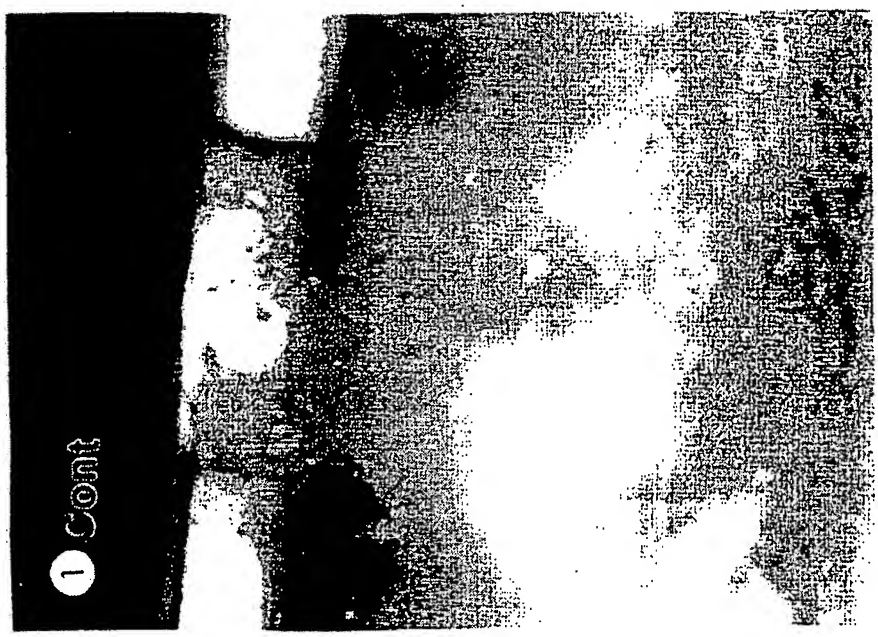
7. The bone regeneration material as claimed in any one of claims 1 to 6, wherein the substrate composed of the material having the biocompatibility is formed into a sheet.

8. The bone regeneration material as claimed in any one of claims 1 to 3, wherein the polyphosphoric acid is contained in a filler for cosmetic surgery.

9. The bone regeneration material as claimed in any one of claims 1 to 8, wherein the polyphosphoric acid is mixed with a bone morphogenetic protein.

10. The bone regeneration material as claimed in any one of claims 1 to 9, wherein the polyphosphoric acid is mixed with a natural substance containing a bone morphogenetic factor.

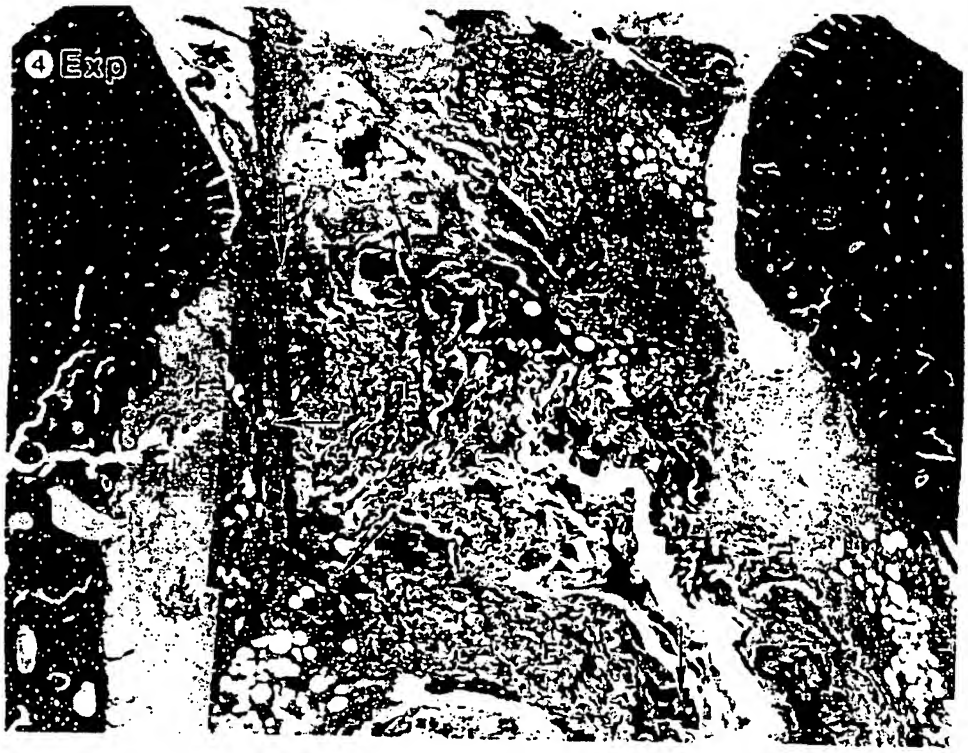
【Fig. 1】



【Fig. 2】

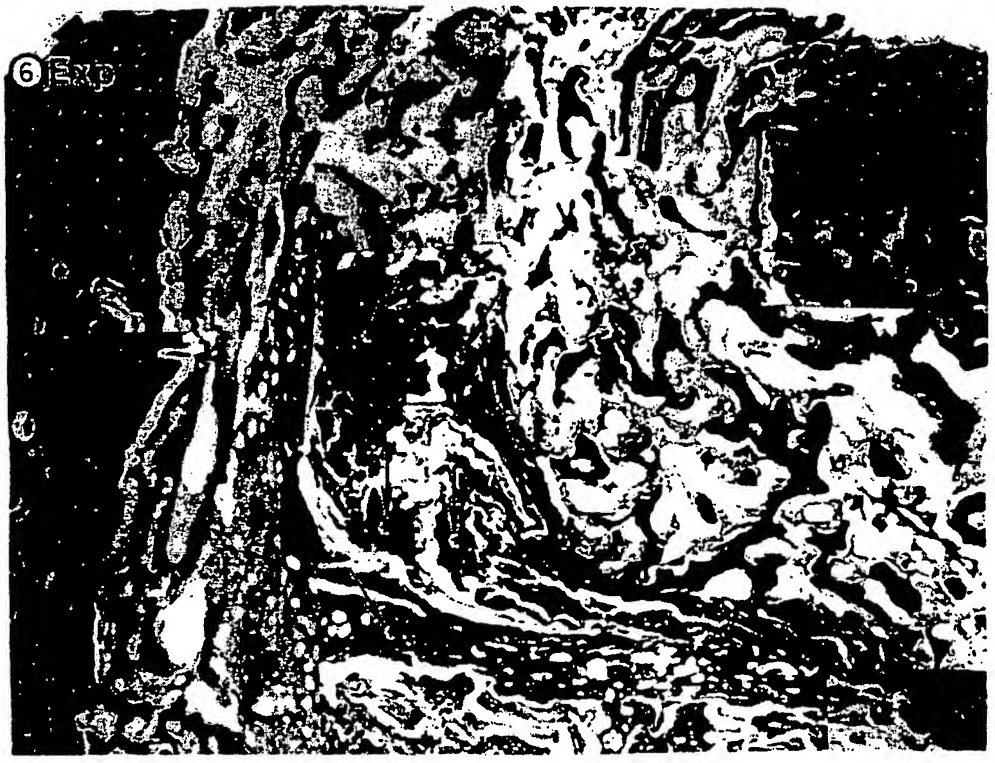
【Fig. 3】

【Fig. 4】



【Fig. 5】

【Fig. 6】



【Fig. 7】

【Fig. 8】

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EUROPEAN SEARCH REPORT

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	17 November 1999	ESPINOSA, M

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	EP 0 347 028 A (NORIAN CORP) 20 December 1989 (1989-12-20)		

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

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ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 99 11 2767

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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(54) Bone regeneration material

Knochenregenerierungsmaterial

Matériau pour la régénération osseuse

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Description

Field of the Invention

[0001] The present invention relates to a bone regeneration material for expediting formation of a new bone tissue. More specifically, the invention relates to a bone regeneration material comprising inorganic polyphosphate.

Description of the Related Art

[0002] A bone is a specialized and hardened connective tissue that is composed of cells and an extracellular matrix, and it is differents from other connective tissue in that matrix of the bone is mineralized. The mineral is calcium phosphate which is a hydroxyapatite crystal ($Ca_{10}(PO_4)_6(OH)_2$). The bone is an extremly hard tissue capable of providing support and protection from physical stress. Accordingly, the reduction or the damage of the bone owing to the fracture or the pathological change leads to the disability, the waste of time and money. When the bone is removed by any reasons, the defected bone need to be generated as soon as possible.

[0003] But if regeneration of the defect cannot occur, replacement of the defect by an artificial bone or by bones from other parts of the body must be operated.

[0004] Further, in the treatment of the damage (fracture) of the bone by the physical shock or the damage of the bone accompanied by the surgical operation, the artificial middle setting of various auxiliary bones including artificial bones and immobilization or fixation of fractured portions of bones have been conducted.

[0005] It takes much time until the bone restores the original shape and function, and the physical and mental stresses of patients are considerably great. Further, the longer the process to healing, the greater the opportunity in which patients might be exposed to bacterial infection. There is a fear that the damaged portions might not be healed completely. Regarding the existing selective materials for increase and regeneration of the bone, various materials which can function as artificial fillers for bone restoration, such as bioceramics, composite materials, bone morphogenetic materials, and natural and synthetic polymers have been studied.

[0006] In case of teeth, replacement and reconstruction of fractured, lost, and any pathologically or physiologically wounded bony parts in the oro-maxillofacial region are also important in many aspect. In particular, an alveolar bone supporting the teeth is vulnerable to bacterial infection. Once the alveolar bone is infected or destroyed, it can hardly be restored to the original level by itself. Generally, implantation for constructing an artificial tooth by inserting a metallic implant member in which titanium is used as a base material into a jaw bone is useful with respect to the loss of an inherent tooth. However, this implantation technique is unsatisfactory for, supporting the portion of the body around the implanted part, and causes an excess occlusal force to the adjacent bony structure. Thus. this has not always been conducted successfully.

As a curing method to solve these problems, there is a method for accelerating regeneration of a tissue and a bone. In order to accelerate regeneration of a bone in a defected bony area, a demineralized bone, hydroxyapatite or the other implant substitute has been used. However, no satisfactory effect has been provided.

[0007] JP-A-1993-0177947 (corresponding to Derwent abstract No. 1995-109588) describes a bone substitute material comprising a shaped piece formed from a powder of tri:calcium alpha phosphate and tetra:calcium phosphate, hardened with a curing liquid, with a bio-absorbable polymeric material at least on the surface, the CaIP ratio being 1.5 to 2.0. Preferred polymers comprise a natural polypeptide, polyglycoside, polyester or polyphosphate. This document does not disclose the use of linear condensed polyphosphoric acids for expediting the formation of new bone tissue.

[0008] EP-A-0 555 807 describes the preparation of a bone substitute material by firing and pulverizing animal bones into animal bone powder, mixing the animal bone powder and a divalent metal compound into a powdery mixture, and kneading the powdery mixture with chitosan sol. Alternatively, a bone substitute sheet is prepared by mixing at least one of apatite and animal bone powder with chitosan sol into a mixture, forming a pre-processed sheet of the mixture, and neutralizing the sheet by an aqueous solution of a compound. The bone substitute material and sheet have a pH value falling within a neutral range.

[0009] Renier and Kohn (Journal of Biomedical Materials Research, Vol. 34, 95-104 (1997)) describe the development and characterization of a biodegradable polyphosphate polymer matrix system as a potential delivery vehicle for growth factors. This document does not relate to linear condensed polyphosphoric acids.

[0010] JP-A-1987-0268124 (corresponding to Derwent abstract No. 1989-169304) describes an artificial tubular-cavity organ comprising a tubular base body of dense material with affinity for tissues whose outer surface is coated with fibrous collagen. This body contains calcium phosphate material, preferably calcium phosphates, such as beta-tricalcium, phosphate and hydroxyapatite. In one example, the said collagen was coated with a diluted solution of polyphosphoric acid. This document does not relate to bone generation materials.

[0011] WO 90/12605 discloses a family of composites suitable for use as materials of construction for implantable

medical devices. In the most preferred embodiment, the substrate polymer is an ortho ester polymer formed by the reaction of a ketene acetal having a functionality of two or more with a polyol.

[0012] This document does not relate to linear condensed polyphosphoric acids and their use as bone regeneration materials.

[0013] Richards et at (Journal of Biomedical Materials Research, Vol. 25, 1151-1167 (1991)) describe the evaluation of a series of bisphenol A-based poly(phosphoesters) as degradable biopolymers. This document, too, does not relate to linear condensed polyphosphoric acids.

Summary of the invention

[0014] It is an object of the invention to provide, for overcoming the defects associated with the conventional bone curing means or substitute bone materials, the use of a bone regeneration material which can expedite new bone formation and shorten a time that lapses until the healing or the restoration.

[0015] That is, the invention relates to the use of a bone regeneration material for expediting formation of a new bone tissue, the bone regeneration material containing a linear condensed polyphosphoric acid represented by the formula $(P_nO_{3n+1})H_{(n+2)}$ or/and a polyphosphate. Further, a preferred embodiment of the invention relates to a bone regeneration material for expediting regeneration of a fractured bone wherein a polyphosphate is contained in a substrate composed of a material having a biocompatibility.

[0016] Still further, a preferred embodiment of the invention relates to a bone regeneration material for expediting formation of a new bone tissue wherein a polyphosphoric acid is contained in a filler for cosmetic surgery.

[0017] Furthermore, a preferred embodiment of the invention relates to a bone regeneration material wherein a polyphosphoric acid is mixed with a bone morphogenetic protein or/and a natural substance containing a bone morphogenetic factor.

Brief Description of the Drawings

[0018]

Fig. 1 is a stereoscopic microphotograph of a hole of a thighbone after one week of the treatment with a collagen tape immersed in sterile water.

Fig. 2 is a stereoscopic microphotograph of a hole of a thighbone after one week of the treatment With a collagen tape immersed in a polyphosphoric acid aqueous solution.

Fig. 3 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after one week of the treatment with a collagen tape immersed in sterile water.

Fig. 4 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after one week of the treatment with a collagen tape immersed in a polyphosphoric acid aqueous solution.

Fig. 5 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after two weeks of the treatment with a collagen tape immersed in sterile water.

Fig. 6 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after two weeks of the treatment with a collagen tape immersed in a polyphosphoric acid aqueous solution.

Fig. 7 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after two weeks of the treatment with a dust bone immersed in sterile water.

Fig. 8 is a microphotograph of histological observation of a state of new bone formation in a hole of a thighbone after two weeks of the treatment with a dust bone immersed in a polyphosphoric acid aqueous solution..

Detailed Description of the Invention

[0019] The polyphosphoric acid used according to the invention is a linear condensed polyphosphoric acid obtained through dehydrocondensation of an orthophosphoric acid, represented by formula $(P_nO_{3n+1})H_{(n+2)}$ and having a structure that two or more PO_4 tetrahedrons are linearly bound with a top oxygen atom held in common. A polyphosphate is a compound having a molecular structure that hydrogen of a hydroxyl group of a polyphosphoric acid is replaced with a metal.

[0020] Examples of the metal include sodium and potassium. n is an integer of at least 2, and it is preferably between 5 and 5,000, more preferably between 15 and 2,000. Examples of the substrate composed of the material having the biocompatibility include a synthetic polymer having a biocompatibility, and a sheet, a film, a fiber and a porous material made of a natural material. The polyphosphoric acid is used by being mixed with these materials, by being coated on the surface of the substrate or by being dipped in fibers or a porous material. Examples of the synthetic polymer include non-bioabsorbable polymers such as polypropylene, polyethylene, polyvinyl chloride, polyester, polycarbonate, cellu-

lose, polyamide, polyfluoroethylene and polyurethane: and bioabsorbable polymers such as polyglycolic acid, polylactide, collagen, polyvinyl alcohol, polyvinyl pyrrolidone. polyamino acid, polycaprolactone, polydioxane and a copolymer of vinyl acetate and an unsaturated carboxylic acid. In the bone regeneration material, the polyphosphoric acid is contained in the material having the biocompatibility. The amount of the polyphosphoric acid contained in the material having the biocompatibility is between 0.001 and 20% by weight, preferably between 0.005 and 10% by weight, more preferably between 1 and 5% by weight. When the amount of the polyphosphoric acid exceeds 20% by weight. cells tend to undergo necrosis. When it is less than 0.001% by weight, the effect of the bone regeneration is decreased.

[0021] Further, the filler for cosmetic surgery is an artificial bone component used as a bone filler in the cosmetic surgery region. Examples of the filler include hydroxyapatite, calcium secondary phosphate, calcium tertiary phosphate and calcium quaternary phosphate. Examples of the bone morphogenetic protein include bone morphogenetic proteins such as BMP-1, BMP-2 and BMP-3, transforming growth factors such as TGF-b, osteopontin and osteocalcin. Further, examples of the natural substance containing a bone morphogenetic factor include crushed animal bone, mineral-defective bone substrate and the like. The natural substance containing a bone morphogenetic factor is mixed with a polyphosphoric acid in combination with the bone morphogenetic protein, and the mixture can locally be administered as an implant or a device. In this case, the product to be administered is occluded or injected in a physiologically acceptable viscous form free from a pyrogenic substance and suitable for feeding into a fractured bone site. Consequently, a hard or soft bone structure is formed in the fractured bone site, providing a matrix which can be re-absorbed into the body in an optimum state.

[0022] The bone regeneration material used according to the invention is used, as an osterogenic preparation containing the polyphosphoric acid, in preventive applications such as improvements of the reduction of the occlusive fracture or the complicated fracture and the fixation of artificial joints. Further, the osterogenic preparation newly induces the bone formation, and it is used in the restoration of the innate or traumatic defective portion or the defective portion caused by tumor incision, and in the treatment of the cosmetic surgery, the treatment of the periodontal disease or the other dental restoration process. Moreover, with respect to the bone regeneration material used according to the invention, the material containing the polyphosphoric acid is used by being coated on the surface of the substrate composed of the material having the biocompatibility, such as a sheet, a film, fibers or a porous material.

[0023] The bone regeneration material of the invention can expedite new bone formation and shorten a time that, lapses until the healing or the restoration in the treatment of bone fracture owing to a physical shock or of damage of a bone accompanied by surgical operation.

Examples

[0024] The present invention is illustrated specifically by referring to the following Examples.

Example 1

[0025] A white rabbit (from New Zealand, body weight 3 kg) was subjected to anesthetic injection, and the thighbone thereof was exposed. Two holes were formed in the neck of the thighbone and near the joint cone, an end of the thighbone using a sterilized drill (3 mm in diameter) until the tip of the drill reached the soft tissue of the thighbone. A collagen tape (CollaTape, supplied by Calcitec) having a size of I cm x 1 cm was immersed in 30 ml of a 2% sodium polyphosphate (average chain length 75) aqueous solution, and sterilely dried. This collagen tape was embedded in the hole formed in the thighbone on the right leg of the white rabbit.

[0026] Further, a collagen tape immersed in sterile water as a control was embedded in the hole formed in the thighbone on the left leg of the white rabbit. In this state, the incised portions were sutured, and the conditions of the new bone generation in the holes of the thighbone after I and 2 weeks were observed. The thighbones extracted for observation were immobilized with 10% formalin.

[0027] Figs. 1 and 2 show states of the holes, after 1 week, which were cut longitudinally, as observed with a stereoscopic microscope. Fig. 1 shows a portion treated with the collagen tape immersed in sterile water. Fig. 2 shows a portion treated with the collagen tape immersed in the polyphosphoric acid aqueous solution. With the collagen tape immersed in sterile water as shown in Fig. 1, no new bone formation was observed at all. Meanwhile, with the collagen tape immersed in the polyphosphoric acid aqueous solution as shown in Fig. 2, it was identified that the new bone in the considerable amount was formed around the bottom edge of the hole, and it was extended to the soft tissue.

[0028] A part of the tissue sample in each hole of the thighbone was taken out for histologically observing the state of the new bone formation in the hole of the thighbone after 1 and 2 weeks using the above-mentioned collagen tapes immersed in sterile water and in the polyphosphoric acid aqueous solution. This tissue sample was treated with 10% EDTA for 2 months to conduct decalcification. The sample decalcified was dehydrated with ethanol at various concentrations and finally with xylene, and wrapped in paraffin. The thus-wrapped sample was cut to a thickness of 5 mm, stained with Azan, and observed under a microscope. With respect to the tissue condition after 1 week, the sample

treated with the collagen tape immersed in sterile water is shown in Fig. 3, and the sample treated with the collagen tape immersed in the polyphosphoric acid aqueous solution in Fig. 4 respectively.

[0029] In the sample treated with the collagen tape immersed in sterile water, as is clear from Fig. 3, the collagen tape (C) was covered with a fibrous tissue (F), and the trabecular bone (TB) derived from the endosteum of the compact bone (B) was approaching to the fibrous tissue. On the other hand, in the sample treated with the collagen tape immersed in the polyphosphoric acid aqueous solution, as shown in Fig. 4, most of the collagen tape was absorbed, and replaced with the fibrous tissue (F). Further, a mass (*) of an immature fibrous trabecular bone derived not from the compact bone (B) but from a new fibrous tissue in the hole could already be identified in six positions of the fibrous tissue.

[0030] Next, with respect to the state after 2 weeks, the sample treated with the collagen tape immersed in sterile water is shown in Fig. 5, and the sample treated with the collagen tape immersed in the polyphosphoric acid aqueous solution in Fig. 6 respectively. In the sample treated with the collagen tape immersed in sterile water, as shown in Fig. 5, both ends of the bone were connected by means of the trabecular bone (TB), but the collagen tape (C) was not completely absorbed, and the trabecular bone (TB) derived from the endosteum surrounded the collagen tape (C) to form a callus. Whereas, the sample treated with the collagen tape immersed in the polyphosphoric acid aqueous solution. as shown in Fig. 6, the collagen tape was absorbed almost completely, and could not be observed. Further, the trabecular bones, primary bones, which were newly formed, were bound to each other, and replaced with the collagen tape. The trabecular bones (*) derived from the new fibrous tissue and replaced with the collagen tape were connected with the trabecular bone derived from the endosteum, and the calluses were formed with the two trabecular bones of the different origins. These restored the holes in such a manner that both ends of the bone were connected therewith.

Example 2

[0031] Holes were formed in the thighbone of the white rabbit in the same manner as in Example 1. Ten milligrams of a human dust bone (particle diameter from 250 to 300 mm) demineralized was immersed in 30 ml of a 2% sodium polyphosphate (average chain length 75) aqueous solution, and sterilely dried. This dust bone was packed in the hole formed in the thighbone on the right leg of the white rabbit. Further, a dust bone immersed in sterile water as a control was packed in the hole formed in the thighbone on the left leg of the white rabbit. In this state, the incised portions were sutured, and the state of the new bone formation in the holes of the thighbone after 2 weeks was histologically observed in the same manner as in Example 1. Fig. 7 shows the results of the electron microscope of the sample treated with the dust bone immersed in sterile water. The hole was mainly filled with the dust bone (DB) and the fibrous tissue (F), and the new trabecular bone was observed only thinly. Fig. 8 shows the results of the electron microscope of the sample treated with the dust bone immersed in the polyphosphoric acid aqueous solution. The hole was filled with the new trabecular bone (*) and the dust bone (DB).

Example 3

[0032] Holes were formed in the thighbone of the white rabbit in the same manner as in Example 1. A collagen tape was immersed in 30 ml of a 2% aqueous solution of sodium polyphosphate (Polyphosphate glass, supplied by Sigma) having various chain lengths, and sterilely dried. The collagen tape was embedded in the hole formed in the thighbone on the right leg of the white rabbit. Further, a collagen tape immersed in sterile water as a control was embedded in the hole formed in the thighbone on the left leg of the white rabbit. In this state, the incised portions were sutured, and the state of new bone formation in the holes of the thighbone after 2 weeks was histologically observed. The chain lengths of sodium polyphosphate used were, in terms of the phosphoric acid group, (I) an average chain length of 15 ($Na_{17}P_{15}O_{46}$), (2) an average chain length of 25 ($Na_{27}P_{25}O_{76}$), (3) an average chain length of 35 ($Na_{37}P_{35}O_{106}$) and (4) an average chain length of 65 ($Na_{67}P_{65}O_{196}$).

[0033] In the experiments using all of polyphosphoric acids, the new bone formation was accelerated without being influenced with the chain lengths of the polyphosphoric acids.

Example 4

[0034] To observe a direct effect of polyphosphate on an osteogenic cell which involves in bone formation, cell activity of MC3T3-E1 osteogenic cell originated from a mouse calvarium was determined by MTT(3-[4,5-dimethylthyazol-2-yl]-2,5-diphenyltetrazoium bromide) assay in the presence of polyphosphate with a chain length of 75 at various concentrations.

[0035] First, the osteogenic EI cell was grown in α-Minimal Essential Medium (α-MEM: Gibco, U.S.A.) supplemented with 10% fetal bovine serum(FBS)at 37°C with 5% CO_2. The grown cells were distributed in each well of a 24-well plate, adjusting the cell number to 5×10^4 cells per ml and incubated in the same medium for 1 day. The cells were

further incubated in the medium without FBS overnight, undergoing their Go stage. After removing the cultured medium, the cells were incubated with increasing concentrations (final concentrations of 0.001~0.01%) of polyphosphate in a total of 1 ml of α -MEM for 24 hours. Instead of polyphosphate, 10 μl of distilled water or α-MEM with 10% FBS was included in the culture as control. After, discarding the culture supernatant, 450 μl of α - MEM without FBS and 50 μ l of MTT(50 mg/ml: Acros Organics, Belgium) were incubated with the cells for 4 hours at 37°C with 5 % CO_2. The supernatant was removed and then the cells treated with 500 μl of isopropanol containing 0.04N HCl. The resulting mixture was collected and measured its MTT activity colorimetrically at 570nm as compared at 630nm as reference.

[0036] The MTT activity of MC3T3-El osteogenic cell at Go stage was increased by approximately 27% in the presence of polyphosphate at the concentrations of 0.001 ~0.0025% ; it was 30% of the increased activity by FBS which contains a variety of growth factors. The activity was gradually decreased down to the control level as concentrations of polyphosphate increased (Table 1).

Table 1.

MTT activity of MC3T3-El osteogenic cell in the presence of polyphosphate with a chain length of 75		
Activators	Absorbance (A570nm-A630nm)	Relative percent
Distilled water	0.223	100.0
Fetal bovines serum (10%)	0.427	191.5
Polyphosphate (0.001%)	0.282	126.5
Polyphosphate(0.0025%)	0. 284	127.4
Polyphosphate(0.005%)	0. 260	116.6
Polyphosphate(0.0075%)	0. 238	106.7
Polyphosphate (0.01%)	0.222	99.6

Claims

1. The use of a linear condensed polyphosphoric acid represented by the formula

$$(P_nO_{3n+1})\,H_{(n+2)},$$

wherein n is an integer of two or more, or/and a metal salt thereof as active ingredient for the manufacturing a bone regeneration material for expediting formation of new bone tissue.

2. The use of claim 1, wherein the polyphosphoric acid or/and polyphosphate is contained in a substrate composed of a material having a biocompatibility.

3. The use of claim 2, wherein the polyphosphoric acid or/and polyphosphate is contained in an amount of from 0.001 to 20% by weight in the material having the biocompatibility.

4. The use of any one of claims 2 or 3, wherein the substrate composed of the material having the biocompatibility is formed into a sheet.

5. The use of claim 1, wherein the polyphosphoric acid or/and polyphosphate is contained in a filler for cosmetic surgery.

6. The use of any one of claims 1 to 5, 'wherein the polyphosphoric acid or/and polyphosphate is mixed with a bone morphogenetic protein.

7. The use of any one of claims 1 to 6, wherein the polyphosphoric acid or/and polyphosphate is mixed with a natural substance containing a bone morphogenetic factor.

Patentansprüche

1. Verwendung einer linearen kondensierten Polyphosphorsäure der Formel

$$(P_nO_{3n+1})\,H_{(n+2)},$$

worin n eine ganze Zahl von zwei oder mehr ist, oder/und eines Metallsalzes davon als aktiver Bestandteil zur Herstellung eines Knochenregenerationsmaterials zur Förderung der Bildung von neuem Knochengewebe.

2. Verwendung nach Anspruch 1, wobei die Polyphosphorsäure oder/und das Polyphosphat in einem Substrat enthalten ist, das aus einem Material mit Biokompatibilität besteht.

3. Verwendung nach Anspruch 2, wobei die Polyphosphorsäure oder/und das Polyphosphat in einer Menge von 0,001 bis 20 Gewichtsprozent in dem Material mit Biokompatibilität enthalten ist.

4. Verwendung nach einem der Ansprüche 2 oder 3, wobei das Substrat, das aus dem Material mit Biokompatibilität besteht, zu einem Flächengebilde geformt ist.

5. Verwendung nach Anspruch 1, wobei die Polyphosphorsäure oder/und das Polyphosphat in einem Füllstoff für die kosmetische Chirurgie enthalten ist.

6. Verwendung nach einem der Ansprüche 1 bis 5, wobei die Polyphosphorsäure oder/und das Polyphosphat mit einem knochenmorphogenetischen Protein vermischt ist.

7. Verwendung nach einem der Ansprüche 1 bis 6, wobei die Polyphosphorsäure oder/und das Polyphosphat mit einer natürlichen Substanz vermischt ist, die einen knochenmorphogenetischen Faktor enthält.

Revendications

1. Utilisation d'un acide polyphosphorique linéaire condensé représenté par la formule

$$(P_nO_{3n+1})H_{(n+2)},$$

dans laquelle n est un entier valant deux ou plus, et/ou d'un sel métallique de celui-ci, en tant qu'ingrédient actif pour fabriquer un matériau de régénération osseuse destiné à la formation accélérée de nouveau tissu osseux.

2. Utilisation selon la revendication 1, dans laquelle l'acide polyphosphorique et/ou le polyphosphate est(sont) contenu(s) dans un substrat composé d'un matériau présentant une biocompatibilité.

3. Utilisation selon la revendication 2, dans laquelle l'acide polyphosphorique et/ou le polyphosphate est(sont) contenu(s) dans une quantité comprise entre 0,001 et 20 % en poids dans le matériau présentant la biocompatibilité.

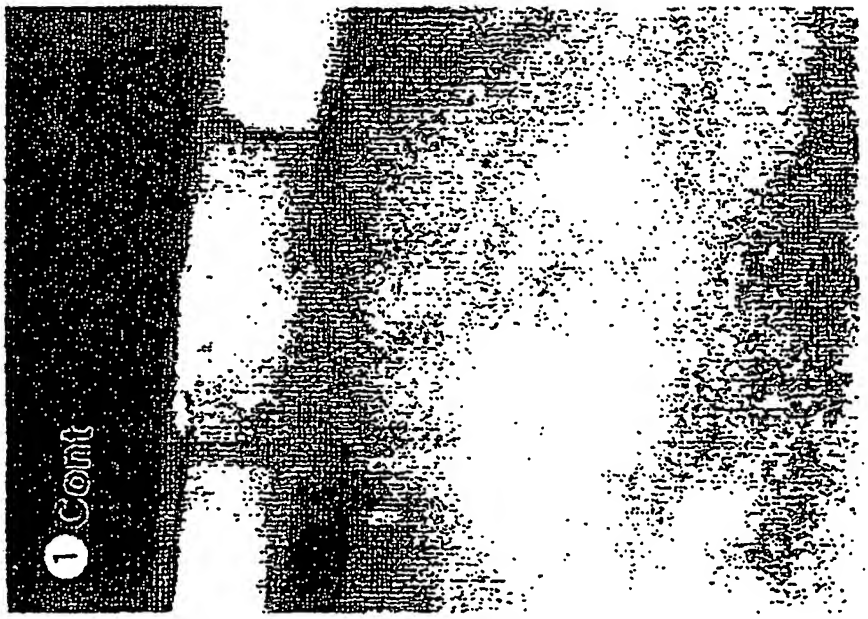
4. Utilisation selon l'une quelconque des revendications 2 ou 3, dans laquelle le substrat composé du matériau présentant la biocompatibilité est formé en une feuille.

5. Utilisation selon la revendication 1, dans laquelle l'acide polyphosphorique et/ou le polyphosphate est(sont) contenu(s) dans une matière de charge pour chirurgie esthétique.

6. Utilisation selon l'une quelconque des revendications 1 à 5, dans laquelle l'acide polyphosphorique et/ou le polyphosphate est(sont) mélangé(s) avec une protéine d'os morphogénétique.

7. Utilisation selon l'une quelconque des revendications 1 à 6, dans laquelle l'acide polyphosphorique et/ou le polyphosphate est(sont) mélangé(s) avec une substance naturelle contenant un facteur osseux morphogénétique.

[Fig. 1]

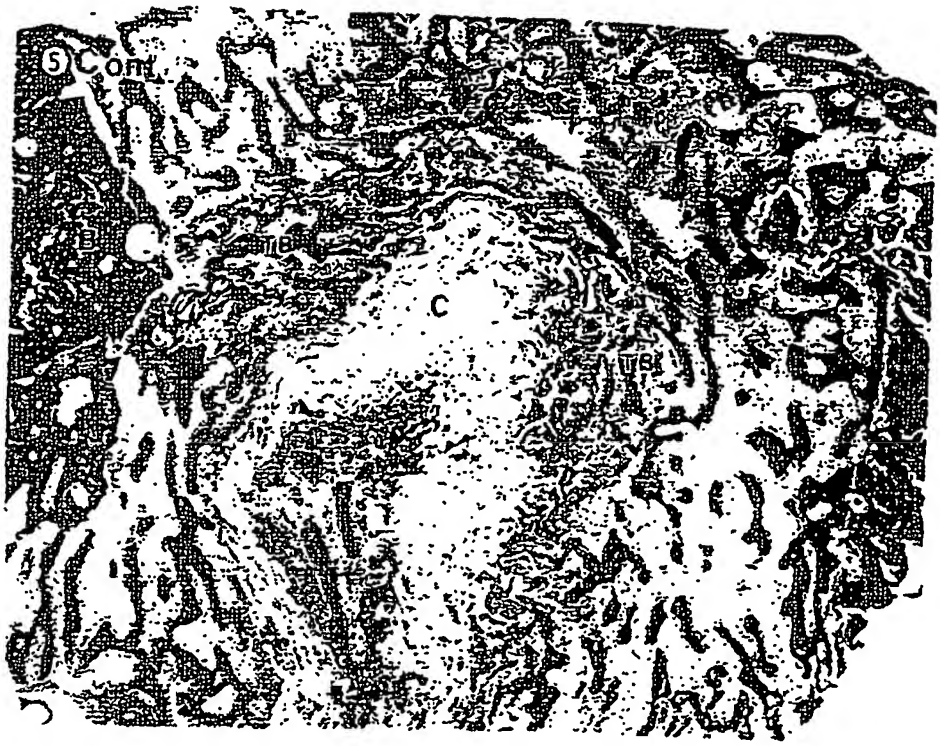


[Fig. 2]

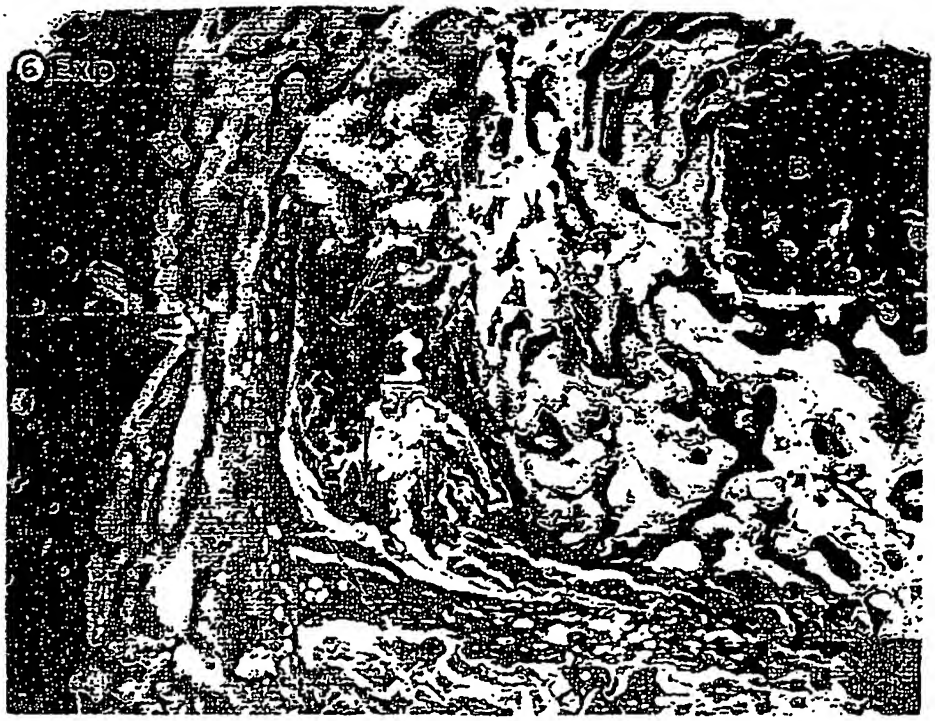
[Fig. 3]

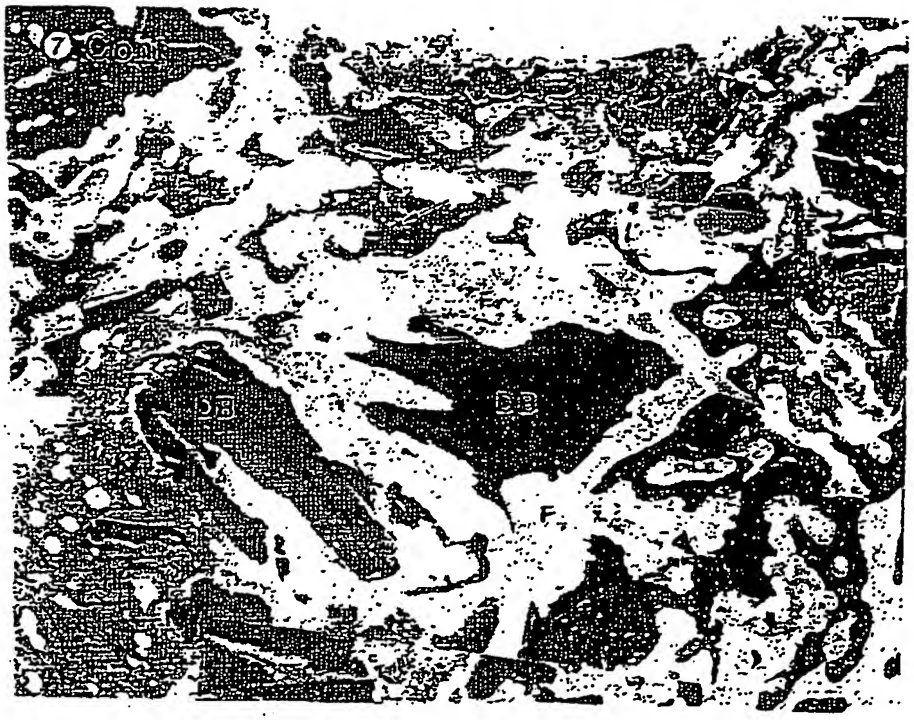
[Fig. 4]

[Fig. 5]



[Fig. 6]

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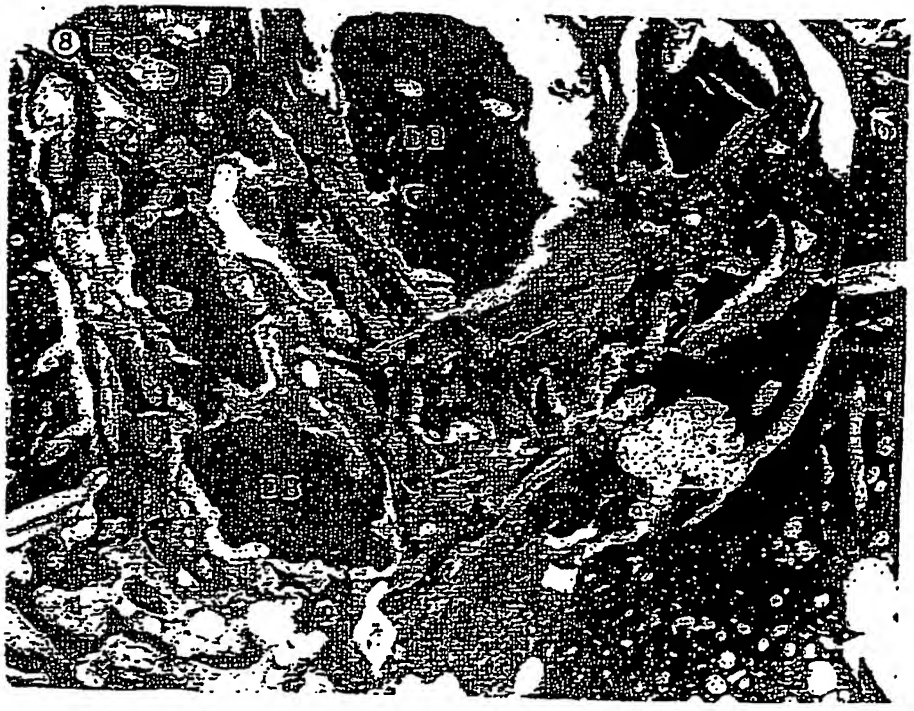
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